Dialysis patients: vulnerable group of patients

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To the Editor: We have read the article by Anderson *et al*,[1] as well as the editorial by Abdel-Kader and Lynn Unruh[2] with great interest and would like to comment.

It is clear from the article by Anderson *et al.* and the editorial that dialysis patients belong to an extremely vulnerable group and that they should get very special attention and care in case of natural disasters.

Our world is not perfect and even today there are wars throughout our planet. These situations are especially threatening for dialysis patients and those with renal transplants.

Unfortunately, Croatian nephrologists had this kind of experience 18 years ago.[3]

There were 31 dialysis units in Croatia in 27 cities providing care for 1819 patients during 1991. When the war started, eight dialysis units with 402 patients had to be evacuated; among them two centers were completely destroyed. Most patients were evacuated without any medical records and a majority of them arrived in Zagreb, the capital of Croatia. As the war escalated, some of the patients were transferred to smaller towns, far from the frontline. In spite of many problems, all patients were treated properly and most of them did not miss the dialysis sessions. This was achieved thanks to the immense efforts of the medical staff and humanitarian aid from all over the word.

Dialysis patients are definitely a very vulnerable and imperiled group, and it is necessary to plan how to take care of them in special circumstances, not just during natural disasters but also in case of unfortunate and tragic wars.

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HIV-1 entry into human podocytes is mediated through lipid rafts

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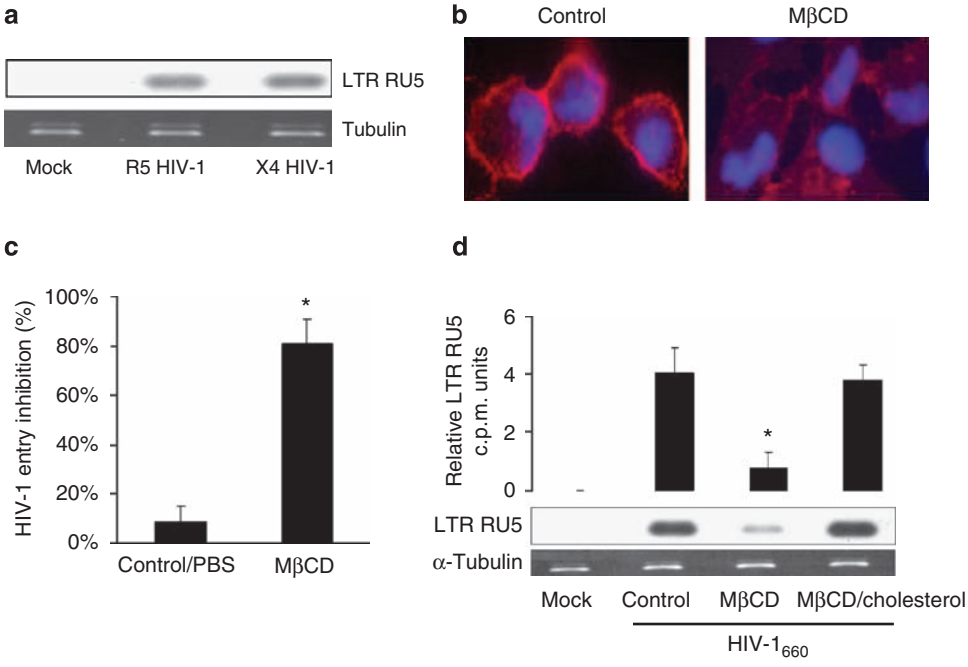
To the Editor: We read with the interest the article by Khan *et al.*[1] Lipid rafts have been reported to have an important role for human immunodeficiency virus (HIV)-1 entry into several cells.[2,3] Renal biopsy studies have demonstrated that HIV-1 infection of renal epithelial cells contribute to the pathogenesis of HIV-1-associated nephropathy.[4,5] As renal epithelial cells do not express classical HIV-1 receptors, their entry into podocytes remains a mystery.[6] We studied the role



of lipid rafts in the entry of HIV-1 into podocytes. Conditionally immortalized human podocytes were exposed to primary strains of HIV-1 with different co-receptor usage—R5 HIV-$1_{92US660}$ and X4 HIV-$1_{92HT599}$. As shown in Figure 1a, human podocytes exposed to either R5 or X4 viral strains were positive for HIV-1-specific strong-stop DNA (LTR RU5).

To determine the role of lipid rafts, we evaluated the effect of methyl-β-cyclodextrin (MβCD) on podocyte HIV-1 entry. MβCD is a derivative of cyclic oligosaccharides and has a lipophilic property that extracts cholesterol from membranes, resulting in lipid rafts disruption.[7] To test the drug effect of MβCD on mock-infected podocytes, we used the cholera toxin B-subunit, a specific marker for the lipid rafts associated GSL GM1.[8] MβCD treatment decreased approximately 70% podocyte cholesterol expression (Figure 1b). As shown in Figure 1c, podocyte viral accumulation significantly decreased after MβCD treatment when compared with the mock-treated control. As cholesterol-replenished cells (first exposed to cyclodextrin and then replenished with exogenous water-soluble cholesterol) showed comparable levels of viral entry (as observed in untreated cells), it appeared that the inhibition of HIV-1 entry was not due to the potential toxicity of MβCD (Figure 1d). Inhibition of viral entry after cholesterol extraction was independent of viral tropism (data not shown). These results support the notion that cholesterol (maintenance of lipid raft integrity) is required for entry of both X4 and R5 HIV-1.

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Response to 'HIV-1 entry into human podocytes is mediated through lipid rafts'

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The study by Mikulak and Singhal[1] showing the importance of cholesterol in the HIV infection of a human renal podocyte model system provides additional support for the important role of cell membrane 'lipid rafts' in HIV infection. Our studies on the binding of R5 gp120 within the human nephron showed that binding was restricted to tubular epithelial cells. gp120–tubular cell binding could be eliminated by detergent extraction of the sections, suggesting that binding occurred within the non-DRM ('non-raft') fraction. Although podocytes were not specifically identified in our study, the lack of gp120 glomerular binding we found indicates that gp120-mediated HIV–podocyte binding must be significantly less than to renal tubular epithelial cells.[2] The lipid raft requirement for HIV infection has been contested,[3,4] and it may be that the lipid raft requirement varies between HIV target cell types. In addition, our studies indicate that Gb_3 is a resistance factor against HIV infection[5] rather than a mediator of infection. Thus, there is a

Figure 1 | Role of lipid rafts in podocyte HIV-entry. (**a**) Podocytes were exposed to different primary human immunodeficiency virus (HIV)-1 strains: R5 HIV-$1_{92US660}$ and HIV-$1_{92HT599}$. Cells were pulsed with the virus for 24 h followed by washing with phosphate-buffered saline (PBS), and then treatment with 0.05% trypsin at 37 °C for 10 min (to eliminate non-internalized virus). The cells were washed again four times with PBS and then plated for culture. After 24 h, cells were trypsinized again, washed, lysed, and analyzed for HIV-specific LTR RU5 by PCR. One representative experiment out of three is shown. Mock infection served as a negative control. (**b**) Equal numbers of podocytes were incubated in serum-free media (SFM) containing either buffer or methyl-β-cyclodextrin (MβCD, 10 mM) for 30 min. At the end of the incubation period, cells were washed and stained with Alexa Fluor 555–conjugated cholera toxin B-subunit (CTx-B) and DAPI. Subsequently, cells were studied under Zeiss Axiovert (Carl Zeiss, Oberkochen, Germany) 200M with ×40 numerical aperture. The presence of podocyte lipid raft ganglioside GM1 is indicated by red fluorescence, whereas blue fluorescence represents nuclei (DAPI). (**c**) Podocytes were pre-incubated in SFM containing either buffer (Control) or MβCD (10 mM) for 30 min. Subsequently, cells were washed and pulsed with HIV-$1_{92US660}$ (pre-treated with 200 U/ml of RNase-free DNase) for 2 h in SFM. After HIV-1 pulsing, cells were trypsinized, washed, and analyzed for HIV-1-specific LTR RU5 by PCR. The percent of inhibition represents the percent of the ratio of the relative LTR RU5 c.p.m. units between samples treated with MβCD and PBS/Control (means ± s.d., $n = 3$). (**d**) After MβCD, treatment podocytes were reconstituted with water-soluble cholesterol (400 μg/ml) for 2 h, washed, and subsequently incubated in SFM containing HIV-$1_{92US660}$ for 2 h. After HIV-1 pulsing, cells were trypsinized, washed and analyzed for HIV-1-specific LTR RU5 by PCR. One representative experiment out of three is shown for the presence of HIV-1-specific strong-stop DNA. Amplification of the α-tubulin gene was used to control for the amount of DNA. The data represent the means ± s.d. of three independent experiments. Mock served as a negative control. *$P < 0.0001$ compared with control.